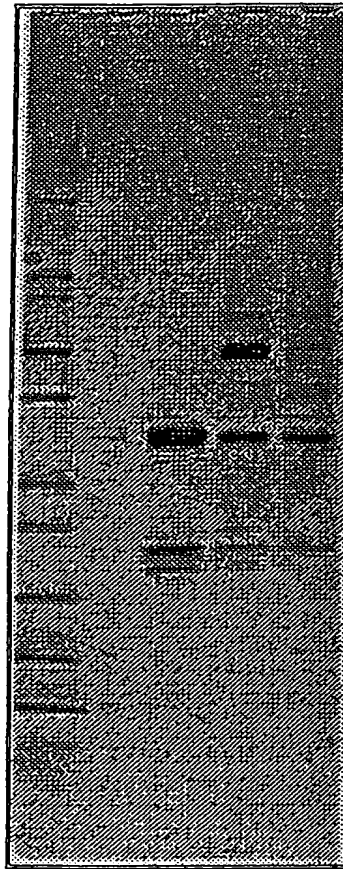


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FIG. 1



M 1 2 3

SDS-PAGE analysis of PEG-modified hA5B7 Fab'

Samples of unmodified hA5B7 Fab' (lane 1), hinge-modified Fab' (lane 2), and randomly-modified Fab' (lane 3) were prepared with non-reducing sample buffer; and 1.5µg of each loaded onto a 4-20% gradient Tris-glycine gel. Standard protein markers (lane M) were also run. These comprised myosin (200kDa), beta-galactosidase (116.3kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.3kDa), glutamate dehydrogenase (55.4kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31kDa), trypsin inhibitor (21.5kDa), lysozyme (14.4kDa), aprotinin (6kDa) and insulin B & A chains (3.5 & 2.5kDa).

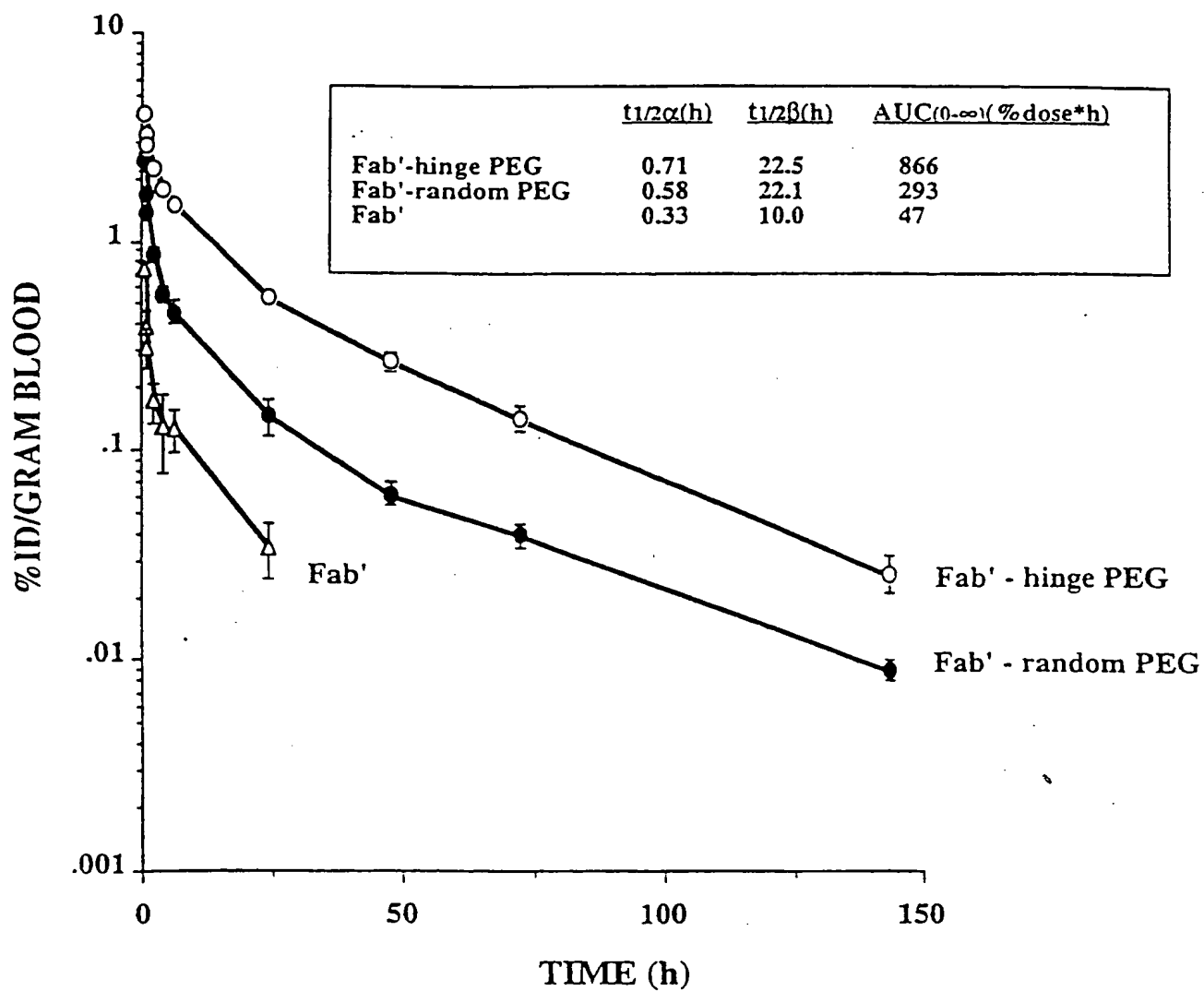
Following electrophoresis, the gel was stained with coomassie blue.

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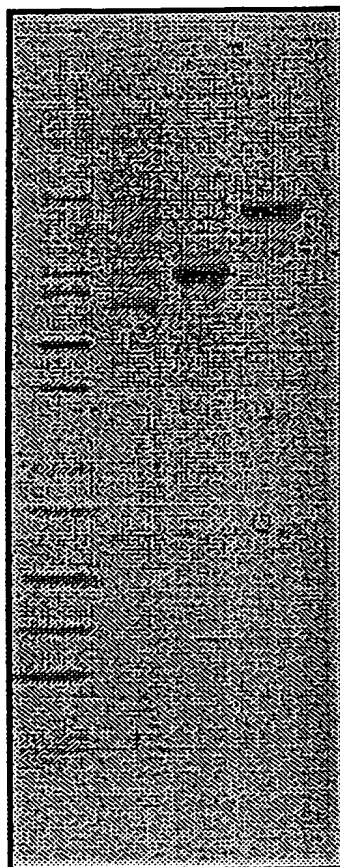
FIG. 2

Pharmacokinetics of 125 -I labelled hA5B7 Fab' in rats



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FIG. 3



M 1 2 3

SDS-PAGE analysis of hTNF40 Fab'-PEG conjugates

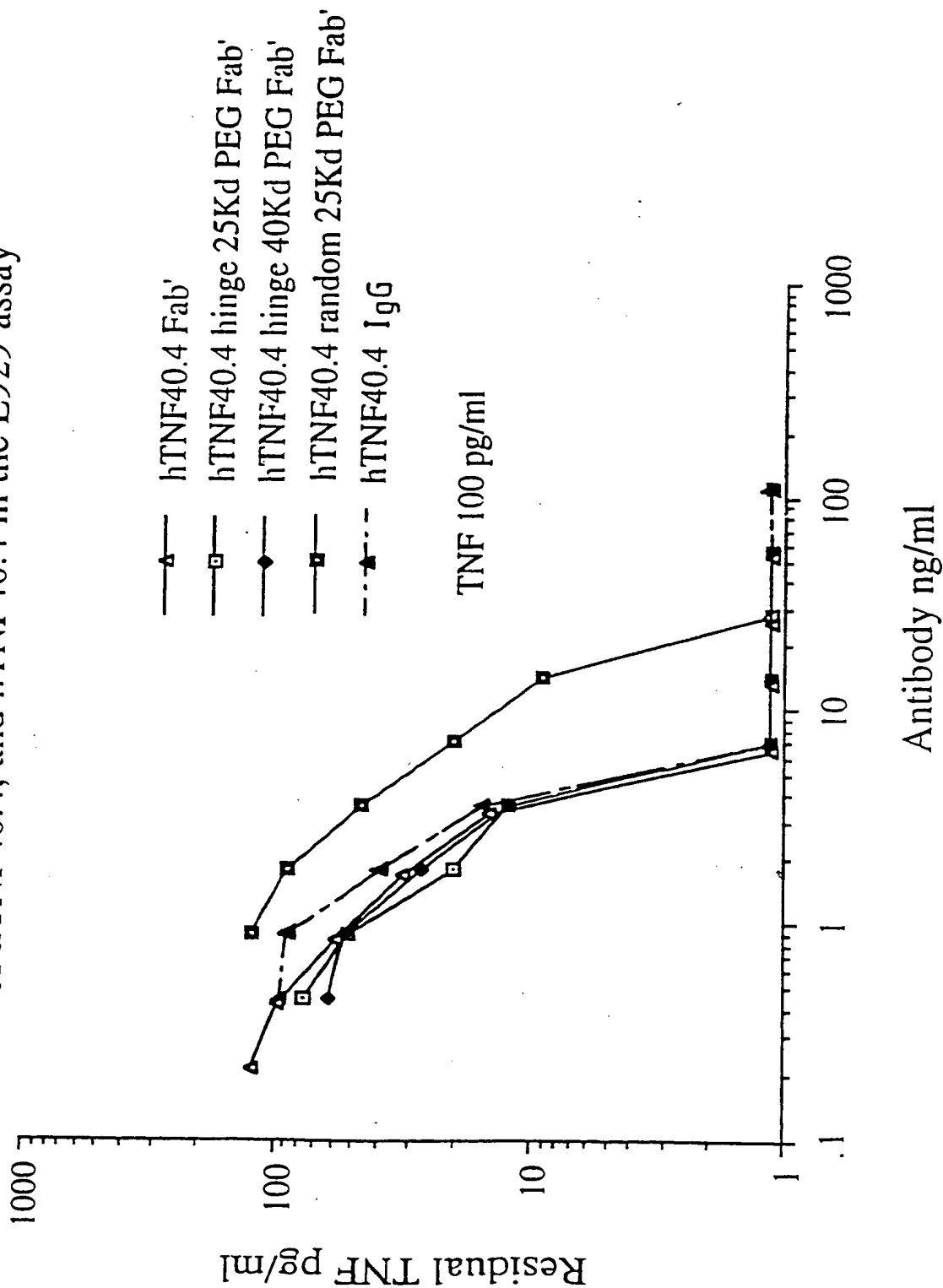
Samples of hTNF40 Fab'-PEG (25kDa) prepared by random conjugation (lane 1), Fab'-PEG (25kDa) prepared by hinge attachment (lane 2), and Fab'-PEG (40kDa) prepared by hinge attachment (lane 3) were prepared with non-reducing sample buffer, and 1.5 μ g of each loaded onto a 4-20% gradient Tris-glycine gel. Standard protein markers (lane M) were also run. These comprised myosin (200kDa), beta-galactosidase (116.3kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.3kDa), glutamate dehydrogenase (55.4kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31kDa), trypsin inhibitor (21.5kDa), lysozyme (14.4kDa), aprotinin (6kDa) and insulin B & A chains (3.5 & 2.5kDa).

Following electrophoresis, the gel was stained with coomassie blue.

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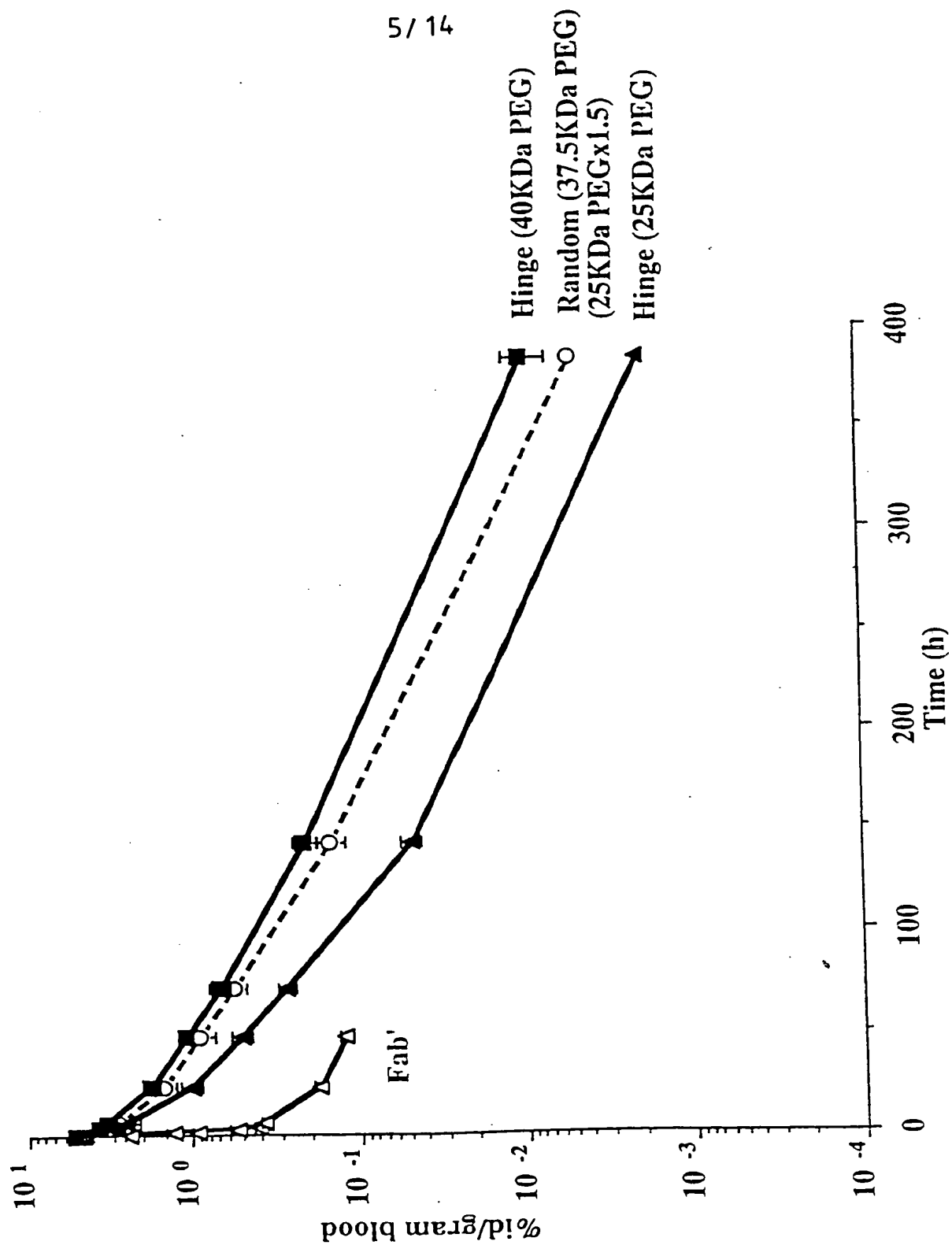
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FIG. 4 Comparison between different PEG Fab' 's, Fab' of hTNF40.4, and hTNF40.4 in the L929 assay



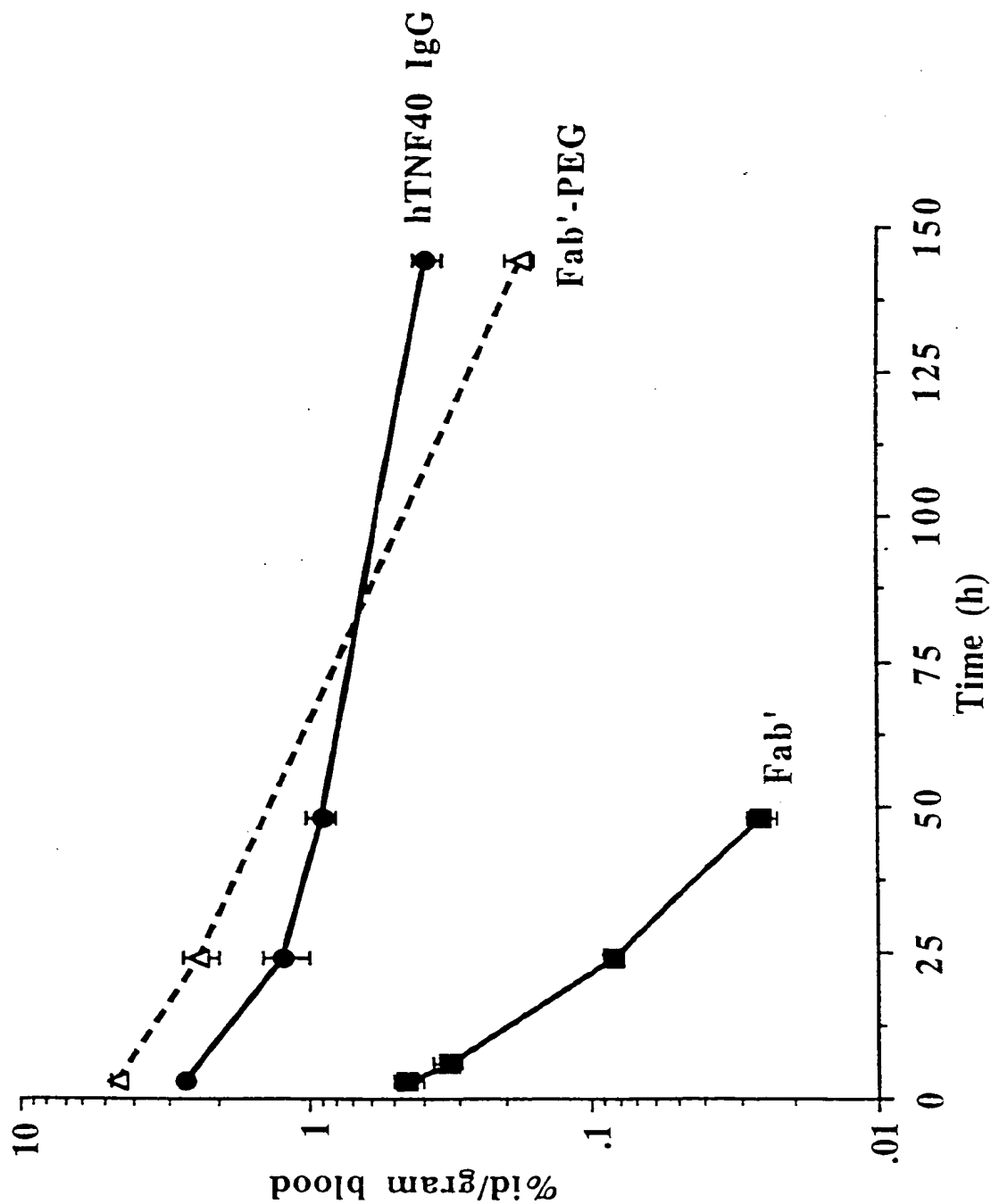
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FIG. 5 Pharmacokinetics of 125 I labelled hTNF40 Fab'-PEG in rats



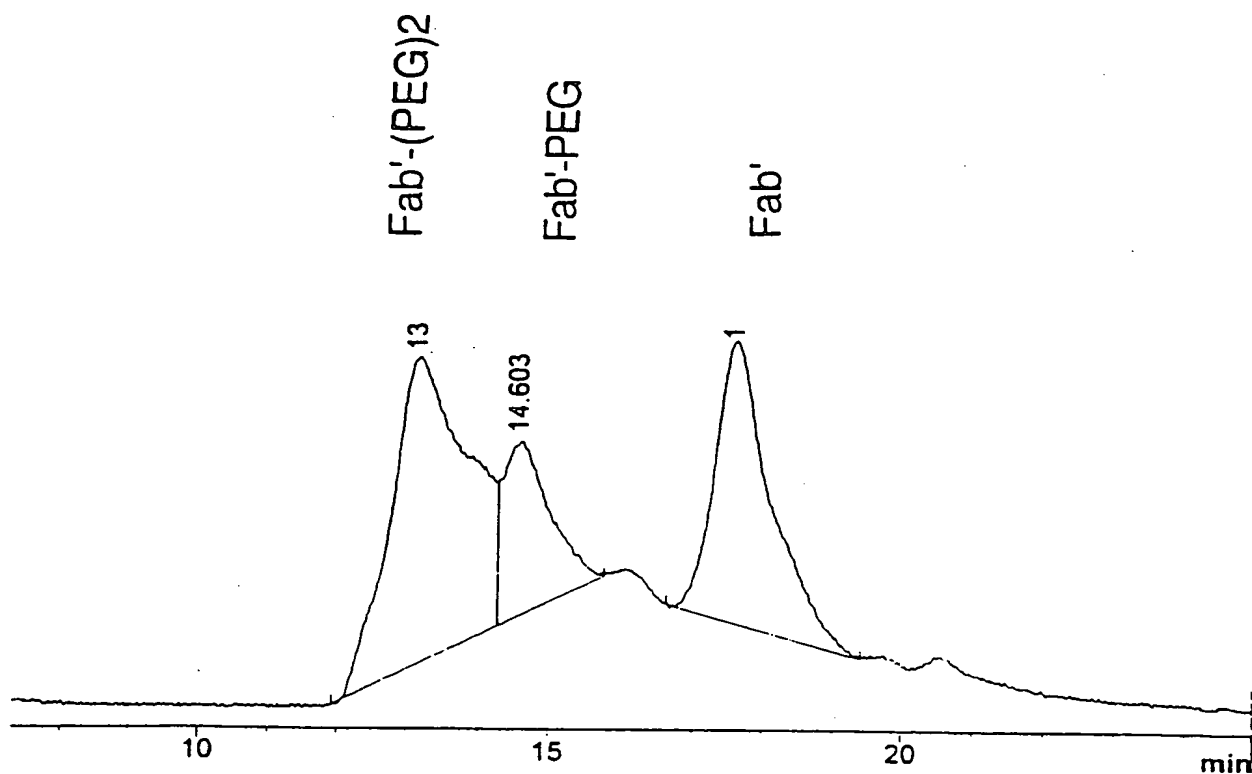
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FIG. 6 Pharmacokinetics of ^{111}In -labelled hTNF40 in rats



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FIG. 7

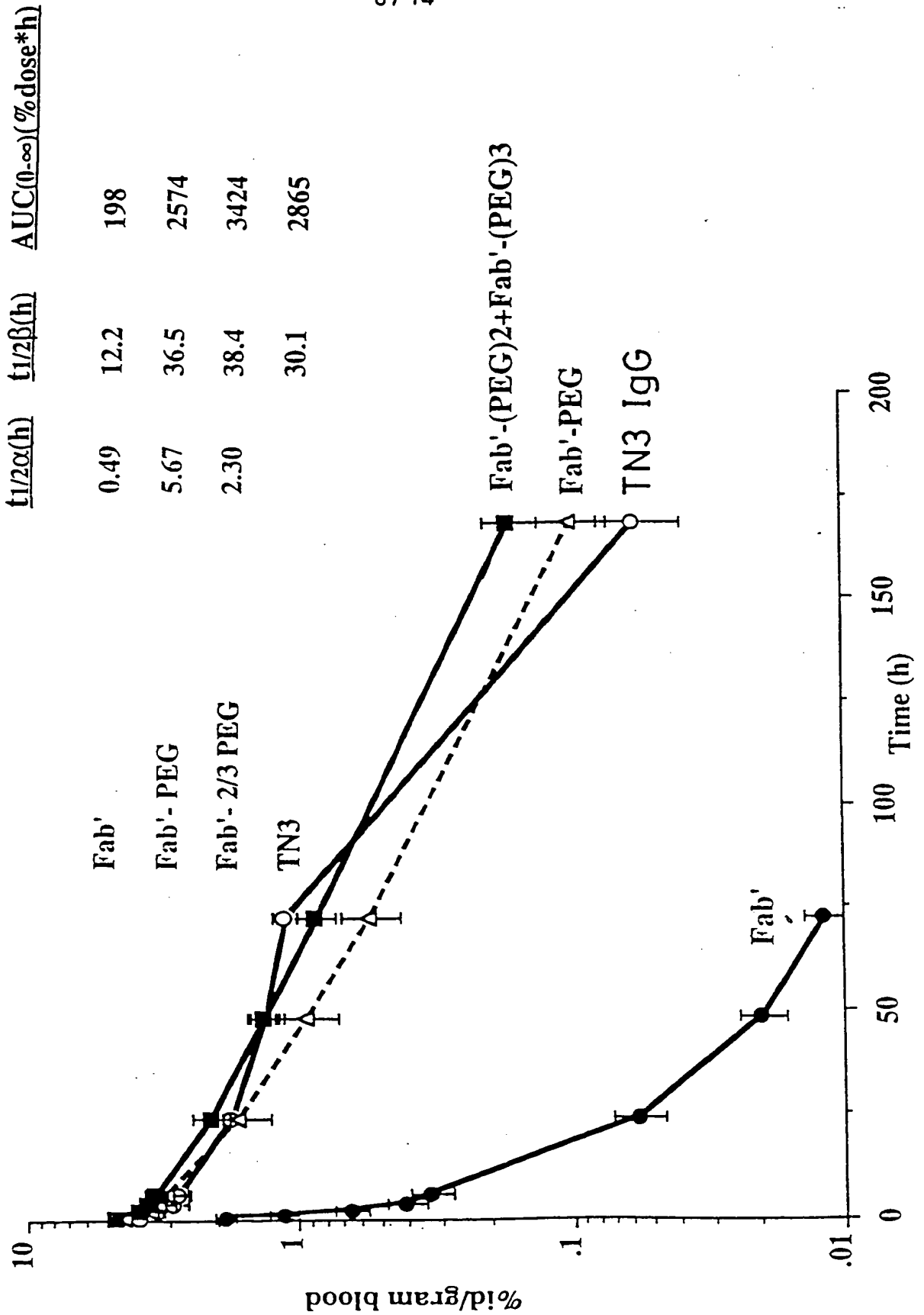


HPLC gel filtration of hTNF40 Fab', Fab'-PEG and Fab'-(PEG)2

DuPont Zorbax GF-250 column run at 1ml/min in 0.2M phosphate buffer pH7.0

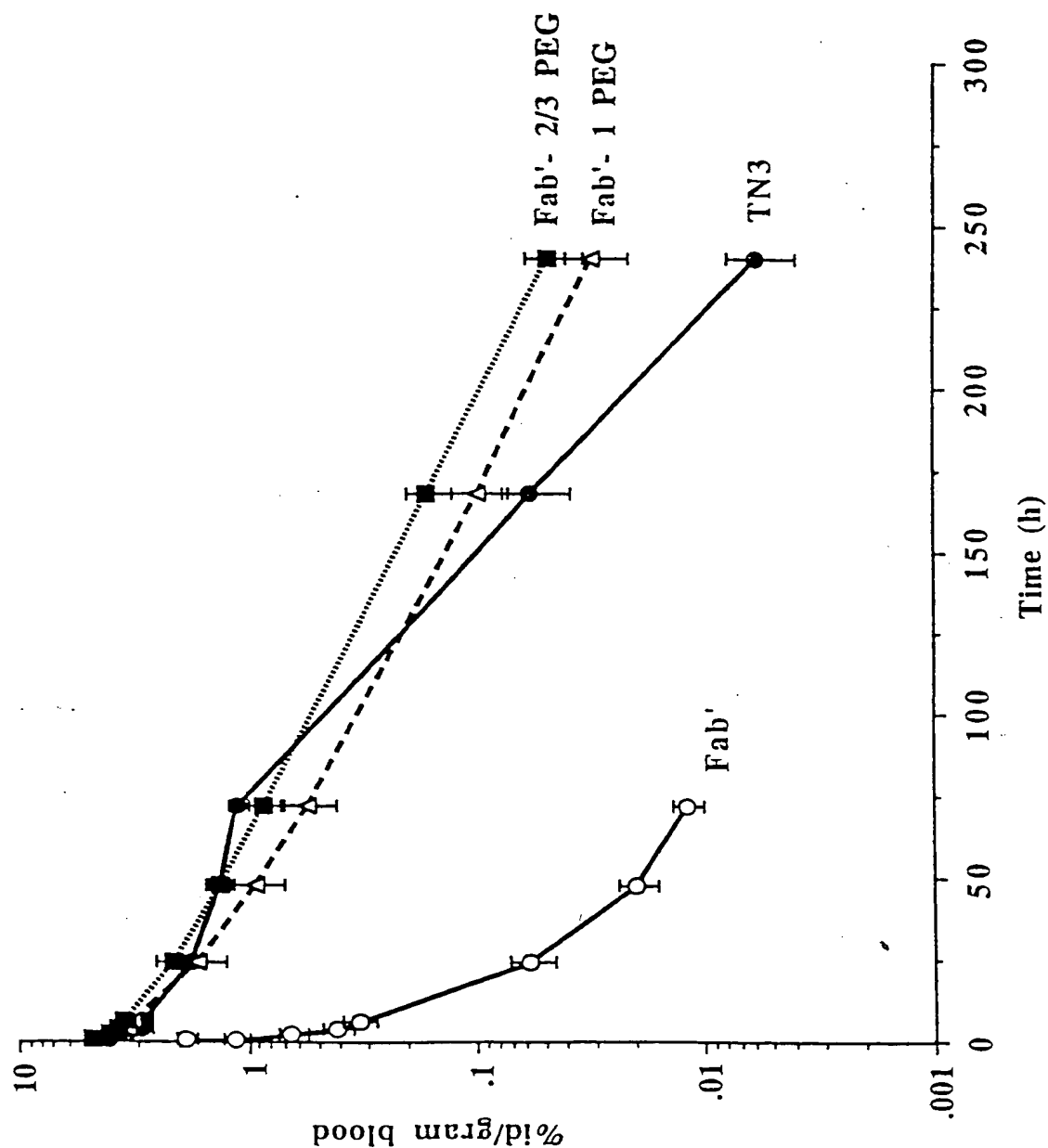
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F/G.8 Pharmacokinetics of 125-I labelled TN3 in rats



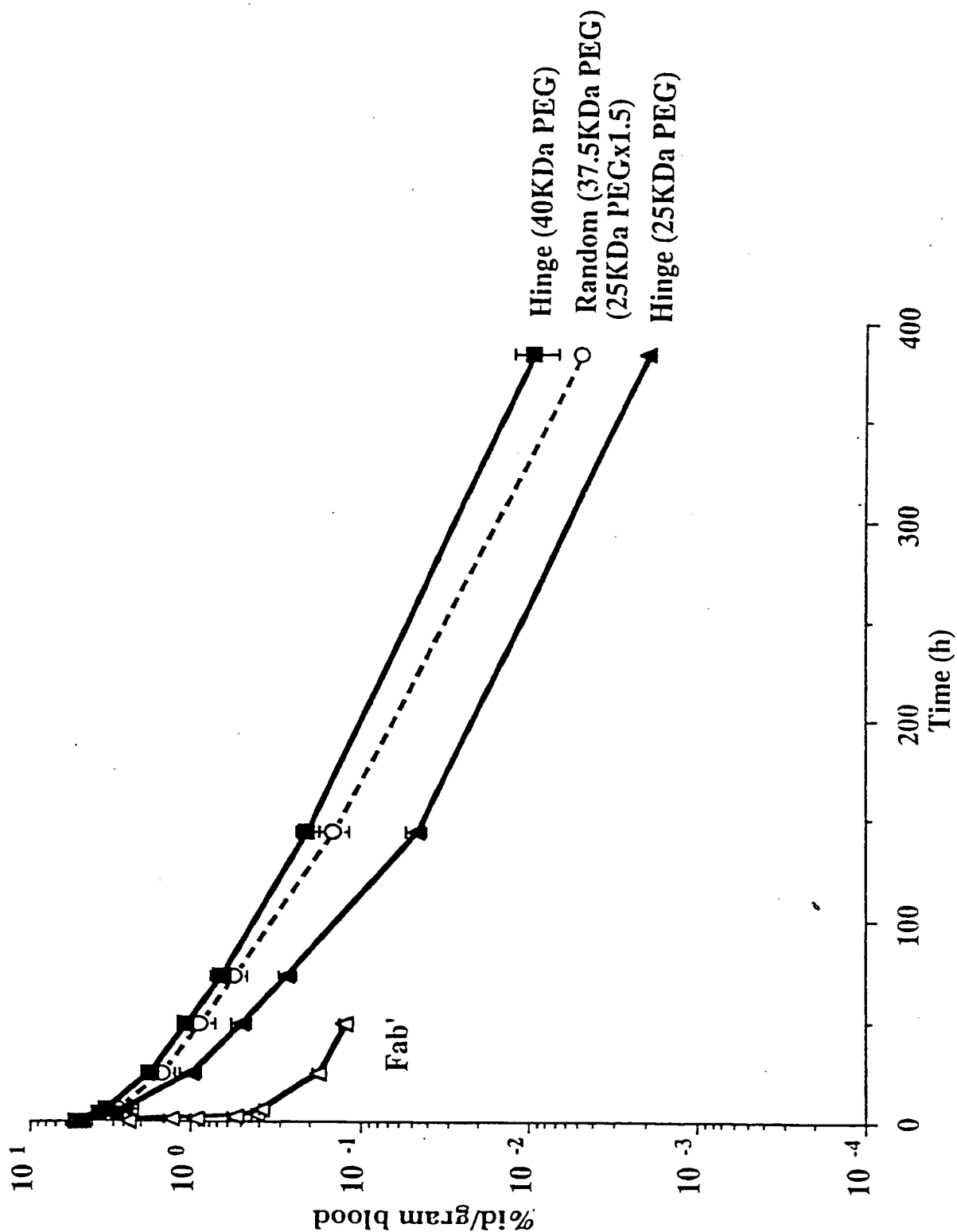
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FIG. 9 ECE15 : Pharmacokinetics of 125-I labelled TN3 in rats
 Fab' PEGylated via the hinge with 25K PEG



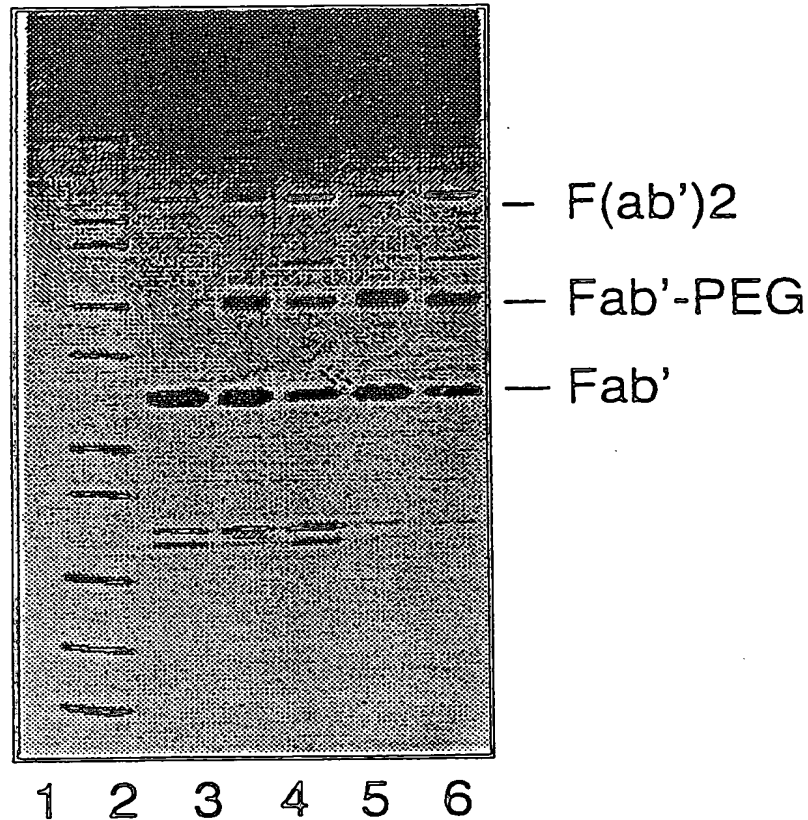
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FIG. 10 Pharmacokinetics of 125 I labelled hTNF40 Fab'-PEG in rats



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SDS-PAGE analysis under non reducing
conditions of Fab'-PEG
(5kDa) prepared using a vinylsulphone or
iodoacetamide reagent



1. Molecular weight marker proteins
2. Purified Fab' (also containing F(ab')₂)
3. Fab'-PEG (5kDa, VS linker) reaction mix
4. Fab'-PEG (5kDa, IA linker) reaction mix
5. Fab'-PEG (5kDa, VS linker) reaction mix
6. Fab'-PEG (5kDa, IA linker) reaction mix

FIG. 11

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FIG. 12

HPLC gel filtration analysis of (a) anti-PDGF β R Fab'-PEG reaction mix showing a peak of Fab'-PEG at 7.7 minutes and a peak of unreacted FAB' at 10.8 minutes.

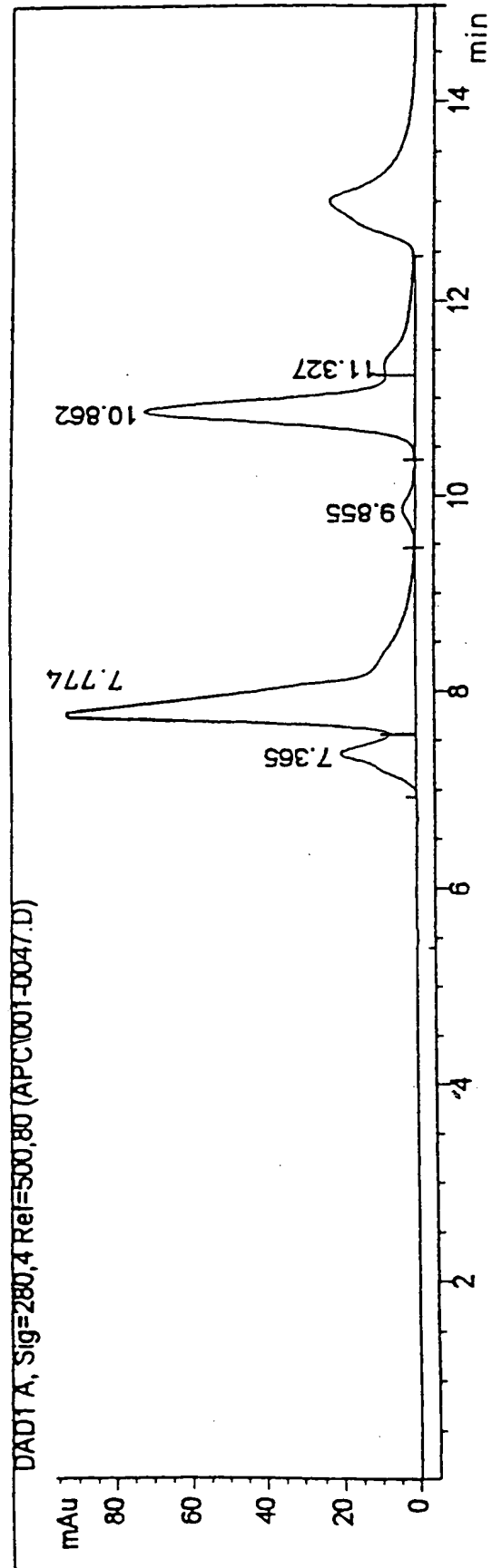
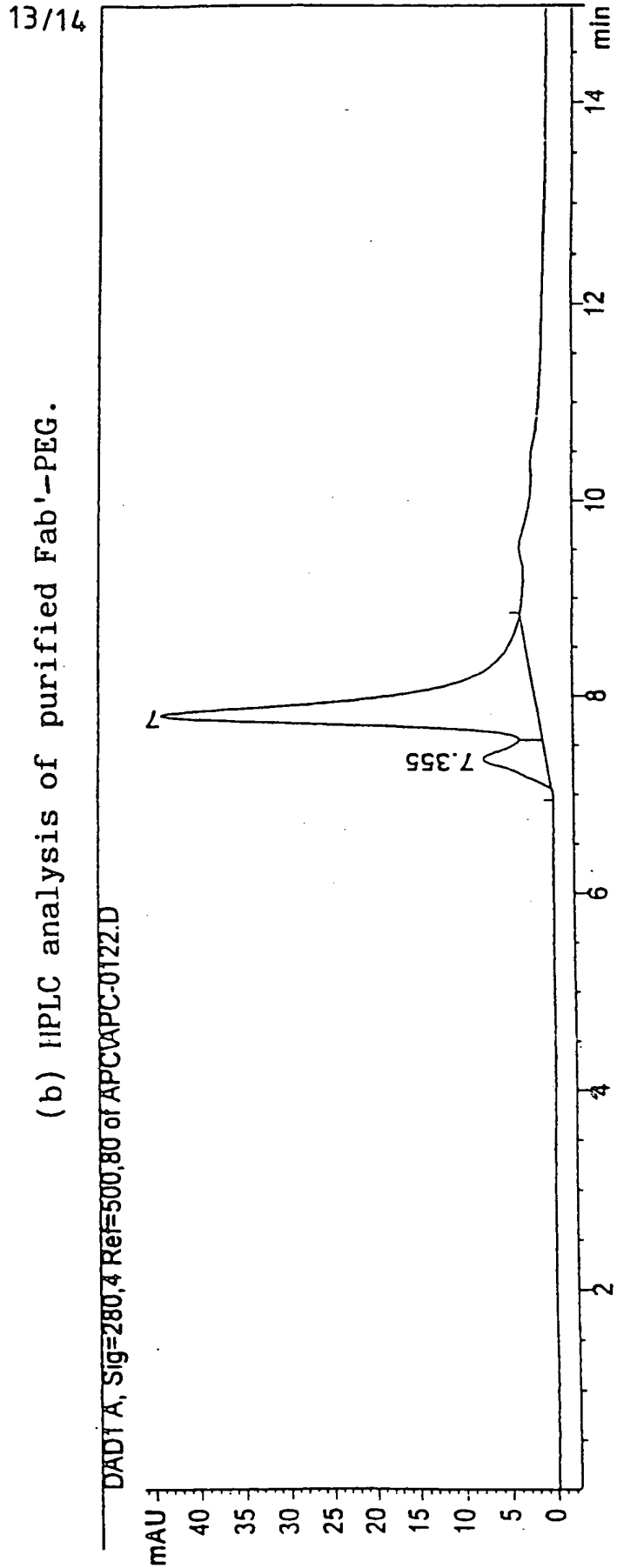


FIG. 12
 (b) HPLC analysis of purified Fab'-PEG.



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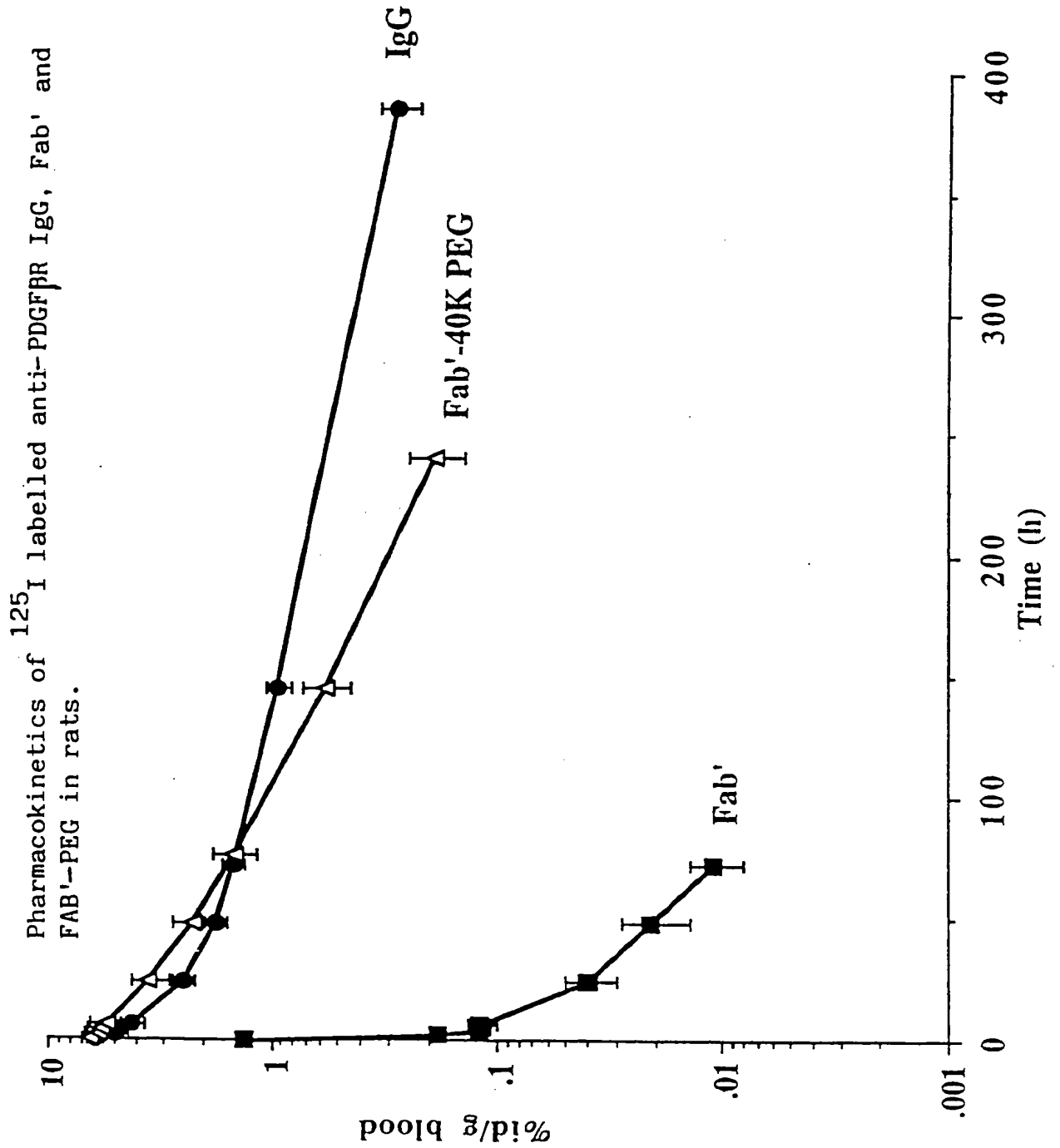


FIG.13